

Effects of External Stimuli on Lymphatic Contractility

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Abstract

The biomechanical principles of the lymphatic system play a key role in the function and efficiency of the lymphatic system yet very little is known about the key biomechanical functions. The goal of this research to take a well-rounded approach into identifying the biomechanical properties of the lymphatic system and the physiological responses of the individual node and the overall vessel. The biomechanical properties of stress and strain will be observed using a biaxial testing device. Axial stretch had a significant effect on several biomechanical characteristics such as amplitude and fractional pump flow. Nitric oxide was also found to have some effect but only at higher concentrations.

Introduction

The lymphatic system plays a key role in maintaining fluid balance and homeostasis within the body alongside the cardiovascular system. While it is closely related to the cardiovascular system there is a key difference in the mechanics of the lymphatic system in that it has no central organ or force driving its unidirectional open-ended flow (Cueni & Detmar, 2006). For a long time the lymphatic system was studied in conjunction with the cardiovascular system and only recently has it been studied as an independent system.

Over the past few decades, the impact of the lymphatic system is most commonly studied in relation to lymphedema, a severe swelling of the extremities caused by an impairment in the lymphatic system such as a ligated vessel. Lymphedema is a complication that often occurs in breast cancer patients after mastectomy when some auxiliary lymph nodes are removed along with the mast. It is estimated that 30-47% of women who undergo a mastectomy with auxiliary

node dissection will develop lymphedema (Shah & Vicini, 2011). One subfield focuses on the development of lymph vessels and how different biological markers may affect lymph growth and regeneration. Genetically modified mouse models have helped identify mutations that cause lymphedema in mice and humans (Schulte-Merker, Sabine, & Petrova, 2011). Other research has shown using mouse models that the growth factor vascular endothelial growth factor C, a commonly studied growth factor in the vascular system, could stimulate lymph regeneration but had no overall effect on curing lymphedema (Uzarski et al., 2008). These advances have led to many insights about the morphogenesis of the lymphatic system but fall short of creating a comprehensive understanding of the lymphatic system.

Research on the biomechanics of the lymphatic system is limited. One area that is especially lacking is the understanding of the function of the individual lymph node and the lymph vessel in a healthy and impaired state. Understanding the underlying biomechanics will result in fundamental knowledge that may lead to a new approach into how we treat diseases of the lymphatic system such as lymphedema. If we understood the lymphatic system to the extent we do the cardiovascular system, we could create more specialized treatments for lymphedema and identify more specific risk factors that lead to lymphatic malfunction.

The goal of the current research is to identify how the lymphatic system reacts to different pressures and to compare the microstructure of healthy and ligated lymph vessels. A biaxial testing device is to control the pressure in the vessel and observe the response. Basic microscopy images can be taken during this phase of testing. Combined, these tests can elucidate basic biomechanical properties of the lymphatic system. The second phase will focus on studying the microstructural composition of the lumen. Immunohistochemistry will be used to isolate different structures and proteins to analyze their contribution to the healthy and

diseased lymphatic vessel. Overall the goal of this research is to provide a holistic picture of the effect of impairment on a lymphatic vessel and find correlation between the tissue composition and biomechanical properties.

Methods and Materials

Lymphatic vessels were excised from rats on the day of euthanization. The vessels were immediately cannulated and connected to a biaxial testing device. Vessels were allowed to equilibrate for one hour. Vessels were then subjected to a series of increasing stretches, 10% 20% and 30% of original vessel length, at a fixed pressure of 2mmHg. Then stretch was held constant and pressure was increased in three steps, 2 mmHg, 4 mmHg and 6mmHg. Since vessels were also subjected to varying doses of nitric oxide (NO) to evaluate its effect on vessel contractility. Data was processed using a specialized peak-finding algorithm in MATLAB and graphed in Prism.

After biaxial testing was completed, the vessel was fixed and allowed to sit for 18 hours. After the fixation period the vessels were washed with 1X PBS for 10 minutes then placed into a cubical container half filled with OCT. An arrow indicating the orientation of the vessel had previously been drawn on the bottom of the container and the vessel was aligned with this arrow. The container was then placed in a -80C freezer for several hours until the OCT had frozen. The remainder of the container was filled with OCT and frozen for up to one month for future studies.

Results

Biaxial testing demonstrated that pressure, stretch and nitric oxide all had a measurable effect on vessel contractility. Stretch had a significant effect on contraction frequency, ejection fraction and fractional pump flow. As stretch increased ejection fraction and fractional pump flow decreased drastically demonstrating the significant effect of axial stretch on contractility (Figure 1).

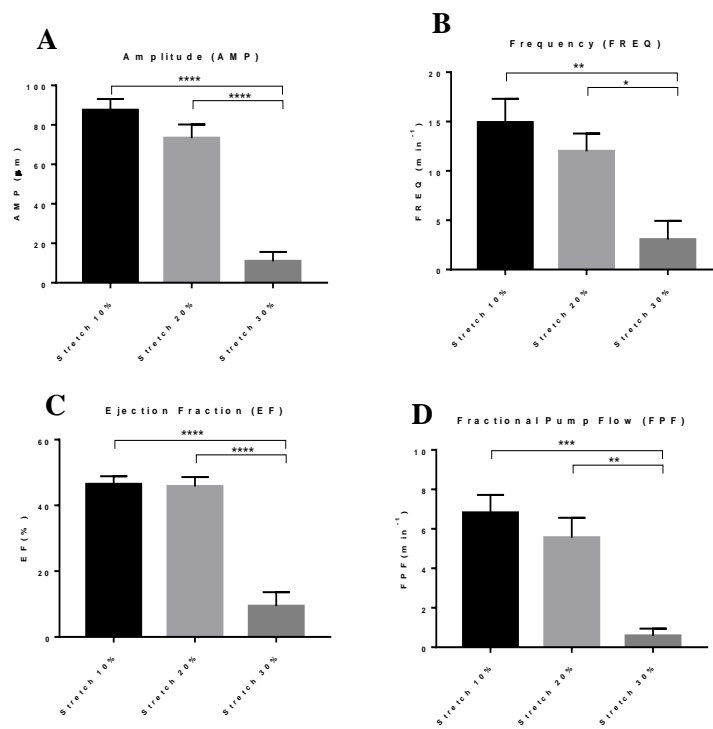


Figure 1. Axial stretch significantly affects biomechanical properties of lymphatic vessel. A) Contraction amplitude is significantly diminished with increasing axial stretch(**** $p < 0.00001$). B) Axial stretch results in decreased frequency of contraction (* $p < .01$, ** $p < 0.001$). C) Ejection fraction was significantly lower at 30% stretch compared to 10% and 20% stretch (**** $p < 0.00001$). D) Fractional pump flow steadily decreased with increasing stress and was significantly lowered at 30% stretch (** $p < 0.001$, *** $p < 0.0001$).

Finally, nitric oxide was shown to have a significant effect on the biomechanical properties of lymphatic vessels but only at high concentrations. A concentration of 10^{-7}M only had a significant effect on the fractional pump flow (Figure 2f). Significant changes in contraction frequency and end diastolic diameter were recorded at 10^{-6}M (Figure 2 b&c). Ejection fraction and contraction amplitude were not significantly different until a high concentration of 10^{-5}M (Figure 2 d&e). Tone was the least affected by nitric oxide as there was no significant change until the highest concentration of 10^{-4}M (Figure 2a).

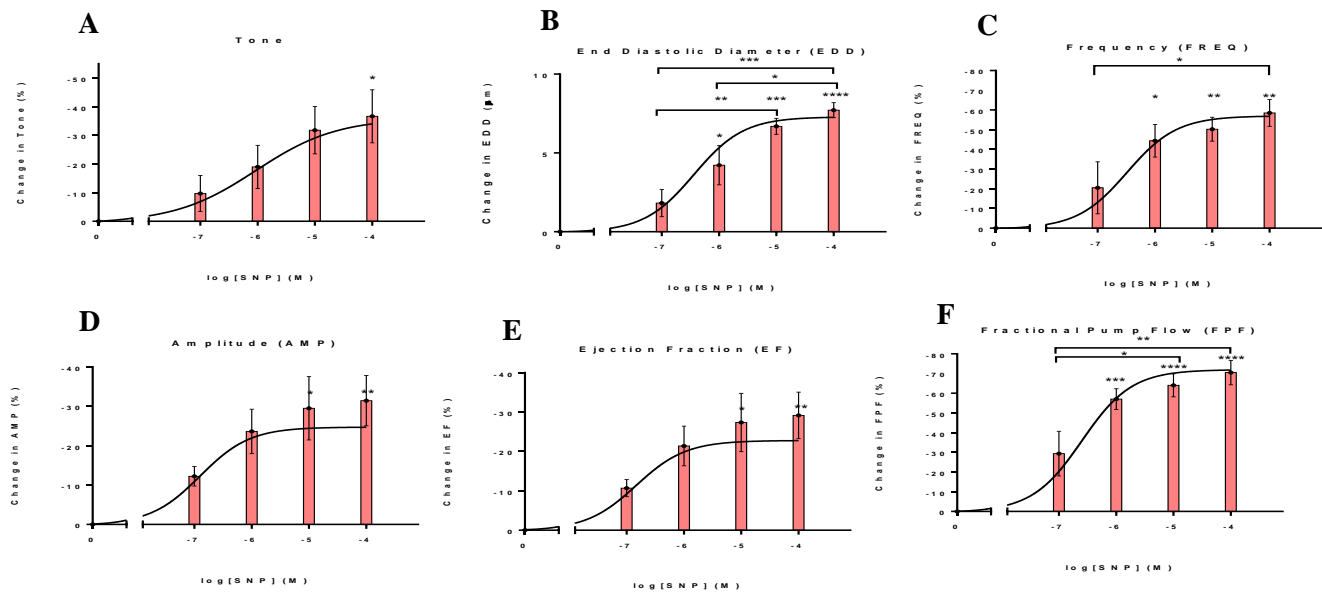


Figure 2. Effect of nitric oxide on the biomechanical properties of rat lymphatic vessel. A) Vessel tone was not significantly different in any samples until 10^{-8} M (* $p < 0.01$). B) End diastolic diameter was significantly different starting at a nitric oxide concentration of 10^{-6} M (* $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$, **** $p < 0.00001$). C) Contraction frequency percentage significantly decreased at concentrations as low as 10^{-6} M (* $p < 0.01$, ** $p < 0.001$). D) Change in amplitude was significantly lower at higher concentrations of nitric oxide (* $p < 0.01$, ** $p < 0.001$). E) At high concentrations ejection fraction significantly changed (* $p < 0.01$, ** $p < 0.001$). F) Fractional pump flow significantly changed starting at low nitric oxide concentrations of 10^{-6} M (* $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$, **** $p < 0.00001$).

Discussion

Research improving the overall knowledge of the lymphatic system can help improve treatments for patients who are suffering from lymphedema as well as improve preemptive diagnosis for the condition.

Current treatment options mainly focus on compression and physical therapy which help improve some of the cosmetic and physical effects of lymphedema but fall short of curing the condition (Brennan & Miller, 1998). The ultimate goal of this project is to create a guideline for the growth and remodeling of the lymphatic vasculature that could cure the complications associated with the debilitating disease. Previous research has demonstrated that this may be a viable option in mice, who naturally have the ability to recover from lymphedema (Mendez, Stroup, Lynch, Waller, & Goldman, 2012).

The significant effects of stretch and nitric oxide on the contractility of the vessel suggests that these may play a role in lymphedema. When part of the lymphatic chain is broken

in lymphedema the rest of the chain must compensate to maintain homeostasis, this may lead to stretching of the vessels or increase in vessel pressure as they carry more lymph per cycle than previously. The effects of nitric oxide also suggest that there may be elevated levels of nitric oxide present in the lymphatic system of lymphedema patients. These results need to be classified further by more animal models as well as patient studies.

In conclusion, there are significant biomechanical and physiological differences between healthy lymphatic vessels and those derived from a rat model imitating the effects of lymphedema. Future studies should evaluate how increased immune response to the site of injury may contribute to lymph and pressure build up in the system. In these studies, the presence of pro-inflammatory molecules should be noted as they can worsen the injury by causing fibrosis buildup. In addition, vessels and skin samples should be fixed and frozen for immunohistochemistry to shed light on the physiological properties with the aim to identify differences in normal and diseased physiology.

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